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CA2205121A1 ☐ **19971113**

Title: (ENG) ANTIADHESIVE PROPERTIES OF POLYACRYLIC ACIDS AND POLYMETHACRYLIC ACIDS

Abstract: The present invention relates to polyacrylic acids and polymethacrylic acids of various molar masses for use as pharmaceuticals and, in particular, for use in the therapy and prophylaxis of diseases associated with excessive cell adhesion, mediated by selectin receptors, in the tissue affected by the disease, for example cardiovascular disorders and rheumatism.

Application Number: CA 2205121 A

Application (Filing) Date: 19970512

Priority Data: DE 19619238 19960513 A X;

Inventor(s): KRETZSCHMAR GERHARD DE ; TOEPFER ALEXANDER DE ; BARTNIK ECKART DE ; HUELS CHRISTOPH DE ; SEIFFGE DIRK DE ; SCHMIDT WOLFGANG DE

Assignee/Applicant/Grantee: HOECHST AG DE

Last Modification Date: 20040303

IPC (International Class): A61K03178

Publication Language: ENG

DE19619238A1 ☐ **19971120** FullText

Title: (GER) Antiadhaesive Eigenschaften von Polyacrylsaeuren und Polymethacrylsaeuren

Application Number: DE 19619238 A

Application (Filing) Date: 19960513

Priority Data: DE 19619238 19960513 A A;

Inventor(s): TOEPFER ALEXANDER DR DE ; KRETZSCHMAR GERHARD DR DE ; SCHMIDT WOLFGANG DR DE ; BARTNIK ECKART DR DE ; HUELS CHRISTOPH DR DE ; SEIFFGE DIRK DR DE

Assignee/Applicant/Grantee: HOECHST AG DE

IPC (International Class): A61K03178

ECLA (European Class): A61K03178

Other Abstracts for This Document: CAN128(01)000374M

EPO Register

EP0807437A1 ☐ **19971119** FullText

Title: (ENG) Polyacrylilic and polymethacrylic acid containing compositions with antiadhesion properties

Abstract: (ENG) Medicaments containing poly(meth)acrylic acid

Polymers (I) of acrylic or methacrylic acid or their salts are claimed for use as medicaments. Also claimed are medicaments consisting of (I) and pharmaceutical auxiliaries.

Application Number: EP 97107625 A

Application (Filing) Date: 19970509

Priority Data: DE 19619238 19960513 A I;

Inventor(s): TOEPFER ALEXANDER DR DE ; KRETZSCHMAR GERHARD DR DE ; SCHMIDT WOLFGANG DR DE ; BARTNIK ECKART DR DE ; HUELS CHRISTOPH DR DE ; SEIFFGE DIRK DR DE

Assignee/Applicant/Grantee: HOECHST AG DE

Last Modification Date: 20041102

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Publication Language: GER
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Other Abstracts for Family Members: CHEMABS128(01)000374M; DERABS C1997-552199
Other Abstracts for This Document: DERABS C1997-552199
Non-Patent Citations:

- NATURE, Bd. 346, Nr. 6283, 2.August 1990, GB, Seiten 425-434, XP000141345T. A. SPRINGER : "Adhesion receptors of the immune system"
- AMERICAN JOURNAL OF PATHOLOGY , Bd. 144, Nr. 3, M{rz 1994, USA, Seiten592-598, XP000573678 A. SEEKAMP ET AL: "Role of Selectins in Local andRemote Tissue Injury following Ischemia and Reperfusion"
- SCIENCE , 23.November 1990, Seiten 1132-1135, XP000601652 G. WALZ ET AL:"Recognition by ELAM-1 of the SIalyl-Lex Determinant on Myeloid and TumorCells"

Patents Cited:

- EP0351987 A X 0
- EP0303296 A X 0
- EP0180435 A X 0
- DE2412090 A X 0
- WO8404680 A X 0

Additional Information:

- Date of Withdrawal of Application 20000927
- Date of request for examination 19980519
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JP10053531A ☑ **19980224** FullText

Title: (ENG) ANTIADHESIVE POLYACRYLIC ACID AND POLYMETHACRYLIC ACID

Abstract: (ENG)

PROBLEM TO BE SOLVED: To provide a method useful for treatment and prevention of diseases accompanying excess cell adhesion derived by selectin receptor by using a polymer of acryclic acid or methacrylic acid.

SOLUTION: Polyacrylic acids and polymers of methacrylic acid or their salts, preferably polyacrylic acids having molecular weight of 900- 63,000g/mol, are effective for treatment and prevention of diseases relating to selectin receptor-induced excess cell adhesion at tissues affected with a disease, particularly diseases of cardiac and vascular system or rheumatic diseases. This medicine is possible to administrate intravenously, orally or nonorally, or as pieces of transplantation and through rectum. The polyacrylic acids and polymethacrylic acids with high molecular weights are extremely strong and specific p-selectin antagonists.

Application Number: JP 12050297 A
Application (Filing) Date: 19970512
Priority Data: DE 19619238 19960513 A X;
Inventor(s): TOEPFER ALEXANDER DR ; KRETZSCHMAR GERHARD DR ; SCHMIDT WOLFGANG DR ; BARTNIK ECKART DR ; HUELS CHRISTOPH DR ; SEIFFGE DIRK DR
Assignee/Applicant/Grantee: HOECHST AG
IPC (International Class): A61K03178; A61K03178; C08F02004
Other Abstracts for Family Members: CHEMABS128(01)000374M; DERABS C1997-552199

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(12) (19) (CA) **Demande-Application**

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(51) Int.Cl.^{6} A61K 31/78
(30) 1996/05/13 (19619238.2) DE

(54) **PROPRIETES ANTIADHESIVES DES ACIDES POLYACRYLIQUES ET POLYMETHACRYLIQUES**

(54) **ANTIADHESIVE PROPERTIES OF POLYACRYLIC ACIDS AND POLYMETHACRYLIC ACIDS**

(57) Acides polyacryliques et polyméthacryliques de masses moléculaires variées à utiliser comme produits pharmaceutiques, en particulier, dans le traitement et la prophylaxie des maladies associées à une adhésion cellulaire excessive, médiée par les récepteurs de la sélectine, dans le tissu affecté par la maladie, par exemple les troubles cardiovasculaires et les rhumatismes.

(57) The present invention relates to polyacrylic acids and polymethacrylic acids of various molar masses for use as pharmaceuticals and, in particular, for use in the therapy and prophylaxis of diseases associated with excessive cell adhesion, mediated by selectin receptors, in the tissue affected by the disease, for example cardiovascular disorders and rheumatism.

Abstract

Antiadhesive properties of polyacrylic acids and polymethacrylic acids

The present invention relates to polyacrylic acids and polymethacrylic acids of various molar masses for use as pharmaceuticals and, in particular, for use in the therapy and prophylaxis of diseases associated with excessive cell adhesion, mediated by selectin receptors, in the tissue affected by the disease, for example cardiovascular disorders and rheumatism.

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Description

Antiadhesive properties of polyacrylic acids and polymethacrylic acids

The present invention relates to polyacrylic acids and polymethacrylic acids of various molar masses for use as pharmaceuticals and, in particular, for use in the therapy and prophylaxis of diseases associated with excessive cell adhesion, mediated by selectin receptors, in the tissue affected by the disease.

The circulation of blood cells, such as, for example, leukocytes, neutrophils, granulocytes and monocytes is a very complex multistage process at the molecular level, only some of the steps in which are known (Review: T.A.Springer, Cell 76, 301-314, 1994).

The most recent research results have shown that the recirculation of lymphocytes which is crucial in immune surveillance, and the localization of neutrophils and monocytes at foci of inflammation obey very similar molecular mechanisms. Thus, in acute and chronic inflammatory processes there is adhesion of the leukocytes to endothelial cells and migration out into the focus of inflammation and into the secondary lymphatic organs.

Numerous specific signal molecules are involved in this process, such as, for example, interleukins, leukotrienes and tumor necrosis factor (TNF), their G protein-coupled receptors and, in particular, tissue-specific cell adhesion molecules, which ensure accurately controlled recognition of immune and endothelial cells. The principal adhesion molecules involved in this, which are to be referred to as receptors hereinafter, include the selectins (E, P and L selectins), integrins and the members of the immunoglobulin superfamily.

The three selectin receptors determine the initial phase of leukocyte

adhesion. E selectin is expressed on endothelial cells a few hours after stimulation by, for example, interleukin -1 (IL-1ß) or tumor necrosis factor (TNF-α) while P selectin is stored in blood platelets and endothelial cells and is presented after stimulation by thrombin, peroxide radicals or substance P, inter alia on cell surfaces. L selectin is permanently expressed on leukocytes but is rapidly eliminated again from the leukocytes during the course of the inflammation.

The adhesion of leukocytes to endothelial cells which is mediated by selectin receptors in the initial phase of inflammatory processes is a natural and necessary immune response to various inflammatory stimuli and injuries to vascular tissue. However, the course of a number of acute and chronic disorders is unfavorably influenced by excessive adhesion of leukocytes and their infiltration into the affected tissue, and by damage to healthy tissue in the sense of an autoimmune reaction. These include, for example, rheumatism, reperfusion injuries such as myocardial ischaemia/infarct (MI), acute inflammation of the lungs after a surgical operation, traumatic shock and stroke, psoriasis, dermatitis, ARDS (adult respiratory distress syndrome) and the restenosis occurring after surgical operations (example angioplasty and by-pass surgery).

The natural ligand has already been used successfully in animal experiments for P selectin-dependent lung injuries (M.S.Mulligan et.al., Nature 1993, 364, 149) and for myocardial reperfusion injuries (M.Buerke et.al., J.Clin.Invest. 1994, 93, 1140).

In screening for biologically active specific antagonists, it has been found, surprisingly, that polyacrylic acids and polymethacrylic acids (commercially available, for example from Fluka) inhibit, depending on their average molar mass, cell adhesion mediated by P selectin.

Accordingly, the present invention relates to a polymer of acrylic acid or of methacrylic acid or their pharmacologically suitable salts for use as pharmaceuticals.

The polymer is preferably polyacrylic acid, preferably its sodium salt.

Particularly suitable for use as pharmaceuticals are polymers of said acids with molar masses of from 900 to 63,000 g/mol.

Said polymers are particularly suitable for producing a pharmaceutical for the therapy or prophylaxis of a disease associated with excessive cell adhesion, mediated by selectin receptors, in the tissue affected by the disease, preferably a cardiovascular disorder or a rheumatic disorder.

The pharmaceuticals according to the invention are generally administered intravenously, orally or parenterally or as implants, but rectal administration is also possible in principle. Suitable solid or liquid pharmaceutical formulations are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, aerosols, drops or injectable solutions in ampule form, and products with protracted release of active substance, for the production of which normally vehicles and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, glidants or lubricants, flavorings, sweeteners or solubilizers are used. Examples of vehicles or ancillary substances which are frequently used are magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, lactalbumin, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents such as, for example, sterile water, alcohols, glycerol and polyhydric alcohols.

The pharmaceutical products are preferably produced and administered in dosage units. Solid dosage units are tablets, capsules and suppositories.

The daily doses necessary for treating a patient differ depending on the activity of the compound, the mode of administration, the nature and severity of the disorder, and the age and body weight of the patient. However, higher or lower daily doses may also be appropriate in some circumstances. The daily dose can be administered either by a single

administration in the form of a single dosage unit or else several small dosage units, or by multiple administration of divided doses at particular intervals. The daily dose to be administered may also depend on the number of receptors expressed during the course of the disease. It is conceivable that only a few receptors are expressed on the cell surface in the initial stage of the disease and, accordingly, the daily dose to be administered is smaller than for patients whose disease is severe.

The pharmaceuticals according to the invention are produced by converting said polymers with conventional vehicles and, where appropriate, additives and/or auxiliaries into the or a suitable dosage form.

The high molecular weight polyacrylic acids and polymethacrylic acids are very potent and specific P selectin antagonists. This can be demonstrated by means of the cell adhesion assay described below.

Example 1:

Primary assays for investigating the effect on cell adhesion by recombinant soluble selectin fusion proteins.

In order to test the activity of the polymers on the interaction between the E and P selectins (old terminology ELAM-1 and GMP-140 respectively) with their ligands, an assay which is specific in each case for only one of these interactions is used. The ligands are offered in their natural form as surface structures on promyelocytic HL60 cells. Since HL60 cells have ligands and adhesion molecules which vary greatly in the specificity, the required specificity of the assay can be provided only by the binding partner. The binding partners used were genetically engineered soluble fusion proteins from, in each case, the extracytoplasmic domain of E or P selectin and the constant region of a human immunoglobulin of subclass IgG1.

Preparation of L selectin-IgG1

Soluble L selectin-IgG1 fusion protein was prepared by using the genetic construct "ELAM-Rg" published by Walz et al., 1990.

For expression, the plasmid DNA was transfected into COS-7 cells (ATCC) using DEAE-dextran (molecular biological methods: see Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Struhl, K. and Smith, J.A. 1990. Current Protocols in Molecular Biology, John Wiley, New York). Seven days after the transfection, the culture supernatant was obtained, centrifuged to remove cells and cell fragments and adjusted to 25 mM Hepes pH 7.0, 0.3 mM PMSF, 0.02% sodium azide and kept at +4°C. (Walz, G., Aruffo, A., Kolanus, W., Bevilacqua, M. and Seed, B. 1990. Recognition bei ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. Science 250, 1132-1135.)

Preparation of P selectin-IgG1

The soluble P selectin-IgG1 fusion protein was prepared by using the genetic construct "CD62Rg" published by Aruffo et al., 1991. The subsequent procedure corresponds to the preparation of L selectin-IgG1 described under A1.

Aruffo, A., Kolanus, W., Walz, G., Fredman, P. and Seed, B. 1991. CD62/-P Selectin recognition of myeloid and tumor cell sulfatides. Cell 67, 35-44.

Preparation of CD4-IgG1

The soluble CD4-IgG1 fusion protein is prepared by using the genetic construct "CD4:IgG1 hinge" published by Zettlemeissl et al., 1990. The subsequent procedure corresponds to the preparation of L selectin-IgG1 described under A1. (Zettelmeissl, G., Gregersen, J.-P., Duport, J. M. Mehdi, S., Reiner, G. and Seed, B. 1990. Expression and characterization

of human CD4: Immunoglobulin Fusion Proteins. DNA and Cell Biology 9, 347-353.)

Procedure for the HL60 cell adhesion assay for recombinant soluble adhesion molecules

1. 96-well microtiter assay plates (Nunc Maxisorb) are incubated with 100 µl of a goat anti-human IgG antibody (Sigma), diluted (1 + 100) in 50 mM Tris pH 9.5, at room temperature for 2 h. Removal of the antibody solution is followed by one wash with PBS.

2. 150 µl of the blocking buffer are left in the wells at room temperature for 1 h. The composition of the blocking buffer is: 0.1% gelatin, 1% BSA, 5% calf serum, 0.2 mM PMSF, 0.02% sodium azide. Removal of the blocking buffer is followed by one wash with PBS.

3. 100 µl of cell culture supernatant from appropriately transfected and expressed COS cells are pipetted into each of the wells. Incubation takes place at room temperature for 2 h. Removal of the cell culture supernatant is followed by one wash with PBS.

4. 20 µl of binding buffer are placed in the wells. The binding buffer has the composition: 50 mM Hepes, pH 7,5;
100 mM NaCl; 1 mg/ml BSA;
2 mM $MgCl_2$; 1mM$CaCl_2$, 3mM $MnCl_2$; 0.02% sodium azide; 0.2 mM PMSF. 5 µl of the test substance are pipetted into this column, mixed by swirling the plate and incubated at room temperature for 10 min.

5. 50 ml of an HL60 cell culture with 200,000 cells/ml are centrifuged at 350 g for 4 min. The pellet is resuspended in 10 ml of RPMI 1640 and the cells are again centrifuged. To label the cells, 50 µg of BCECF-AM (Molecular Probes) are dissolved in 5 µl of anhydrous DMSO; then 1.5 ml of RPMI 1640 are added to the BCECF-

AM/DMSO solution. The cells are resuspended in this solution and incubated at 37°C for 30 min. After centrifugation at 350 g for two minutes, the labeled cell pellet is resuspended in 11 ml of binding buffer, and the resuspended cells are distributed in 100 µl aliquots in the wells of the microtiter plate. The plate is left to stand at room temperature for 10 min in order to allow the cells to sediment to the bottom of the assay plate. During this, the cells have the opportunity to adhere to the coated plastic.

6. To stop the assay, the microtiter plate is completely immersed at an angle of 45° in the stop buffer (25 mM Tris, pH 7.5; 125 mM NaCl; 0.1% BSA; 2 mM $MgCl_2$; 1 mM $CaCl_2$; 3 mM $MnCl_2$; 0.02% sodium azide). The stop buffer is removed from the wells by inversion, and the procedure is repeated twice more.

7. Measurement of the cells adhering in the wells and labeled with BCECF-AM takes place in a cytofluorimeter (Millipore), with a sensitivity setting of 4, an excitation wavelength of 485/22 nm and an emission wavelength of 530/25 nm.

Results:

IC 50 values for P selectin and for E selectin:

	IC 50 (µM, P selectin)	IC 50 (µM, E selectin)
Polyacrylic acid 995 Na salt	165.00	> 2000
Polyacrylic acid 2100 Na salt	125.00	> 2000
Polyacrylic acid 4100 Na salt	21.00	> 2000
Polyacrylic acid 62,900 Na salt	0.009	> 2000

(Specifications according to the information in the Fluka 1996 catalog).

Example 2:

Leukocyte adhesion - test of the activity of the compounds according to the invention in vivo (intravital microscopy on rats):

Tissue destruction by leukocytes which migrate in or block the microcirculation plays a crucial part in inflammatory processes and other cytokine-activating conditions. The first phase, which is crucial for the subsequent disease process, is activation of leukocytes within the blood stream, in particular in the pre- and postcapillary region. This results, after the leukocytes have left the axial flow of blood, in an initial adhesion of the leukocytes to the inner wall of the vessel, i.e. in the vascular endothelium. All the following leukocyte effects, i.e. active migration through the vessel wall and subsequent oriented migration in the tissue, are subsequent reactions (Harlan, J.M., Leukocyte-endothelial interaction, Blood 65, 513-525, 1985).

This receptor-mediated interaction of leukocytes and endothelial cells is regarded as an initial sign of the inflammatory process. Besides the

adhesion molecules which are already physiologically expressed, on exposure to mediators of inflammation (leukotrienes, PAF) and cytokines (TNF-alpha, interleukins) there is sequential massive expression of adhesion molecules on the cells. They are at present divided into three groups: 1. Immunoglobulin gene superfamily, 2. Integrins and 3. Selectins. Whereas adhesion between molecules of the Ig gene superfamily takes place via protein-protein linkages, in the cooperation between selectins it is lectin-carbohydrate linkages which predominate (Springer, T.A., Adhesion receptors of the immune system. Nature 346, 425-434, 1990; Huges, G., Cell adhesion molecules - the key to an universal panacea, Scrips Magazine 6, 30-33, 1993; Springer, T.A., Traffic signals for lymphocyte recirculation and leukocyte emigration; The multistep paradigm. Cell 76, 301-314, 1994).

Method:

The induced adhesion of leukocytes is quantified by a technique of intravital microscopic investigation on the rat mesenterium (Atherton A. and Born G.V.R., Quantitative investigations of the adhesiveness of circulation polymorphonuclear leukocytes to blood vessel walls. J. Physiol. 222, 447-474, 1972; Seiffge, D. Methoden zur Untersuchung der Rezeptor-vermittelten Interaktion zwischen Leukozyten und Endothelzellen im Entzündungsgeschehen [Methods for investigating the receptor-mediated interaction between leukocytes and endothelial cells during inflammation], in: Ersatz- und Ergänzungsmethoden zu Tierversuchen in der biomedizinischen Forschung [Methods for replacing and supplementing animal experiments in biomedical research], Schöffl, H. et al., (Editors) Springer, 1995 (in the press)). Under ether-inhalation anesthesia, prolonged anesthesia is induced by intramuscular injection of urethane (1.25 mg/kg body weight). After vessels have been exposed by dissection (femoral vein for injection of substances and carotid arteries for measurement of blood pressure), catheters are tied into these. The appropriate transparent tissue (mesenterium) is then exposed and transposed to the microscope stage and covered with liquid paraffin at 37°C by standard methods known from the literature (Menger, M.D. and

Lehr, H., A. Scope and perspectives of intravital microscopy-bridge over from in vitro to in vivo, Immunology Today 14, 519-522, 1993). The test substance is administered to the animal i.v. (10 mg/kg). The experimental increase in blood cell adhesion is induced by cytokine activation by systemic administration of lipopolysaccharide (LPS, 15 mg/kg) 15 minutes after administration of the test substance (Foster S.J., McCormick L.M., Ntolosi B.A. and Campbell D., Production of TNF-alpha by LPS-stimulated murine, rat and human blood and its pharmacological modulation, Agents and Actions 38, C77-C79, 1993, 18.01.1995). The increased adhesion, caused thereby, of leukocytes to the endothelium is quantified by direct vital microscopy or with the aid of fluorescent dyes. All the measurement steps are recorded by video camera and stored in a video recorder. The number of rolling leukocytes (i.e. all the visibly rolling leukocytes which are slower than the flowing erythrocytes) and the number of leukocytes adhering to the endothelium (residence time longer than 5 seconds) are measured every 10 minutes for a period of 60 minutes. After completion of the experiment, the anesthetized animals are sacrificed painlessly and without excitation by systemic injection of T61. For the evaluation, the results in each case from 8 treated and 8 untreated animals (control group) are compared (results stated in percent).

Intravital microskopy on rats:

a) Polyacrylic acid (2100 Na salt): dose: 3 mg/kg; administration: i.v.; species: SPRD (m); weight in g:
284+/- 9,6; number of vessels: 15; vessel diameter in µm 28 +/- 2.2; leukocytes in $10^3/mm^3$: 8.2 +/- 2; fibrinogen in mg/100 ml:
96 +/- 10.1; inhibition: -3%

b) Polyacrylic acid (62900 Na salt): dose: 3 mg/kg; administration: i.v.; species: SPRD (m); weight in g:
276 +/- -13.6; number of vessels: 18; vessel diameter in µm 27 +/- 5.2; leukocytes in $10^3/mm^3$: 12.5 +/- 3.5; fibrinogen in mg/100 ml:
101 +/- 12.3; inhibition: 64%.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A polymer of acrylic acid or methacrylic acid or its pharmacologically acceptable salts for use as pharmaceutical.

2. A polymer as claimed in claim 1, wherein the polymer is a polyacrylic acid.

3. A polymer as claimed in claim 2, wherein the polymer is the sodium salt of a polyacrylic acid.

4. A polymer as claimed in any of claims 1 to 3, wherein the average molar mass of the polymer is from 900 to 63,000 g/mol.

5. The use of a polymer as claimed in any of claims 1 to 4 for producing a pharmaceutical for the therapy or prophylaxis of a disease associated with excessive cell adhesion, mediated by selectin receptors, in the tissue affected by the disease.

6. The use as claimed in claim 5, wherein the disease is a cardiovascular disorder.

7. The use as claimed in claim 5, wherein the disease is a rheumatic disorder.

8. A pharmaceutical comprising at least one polymer as claimed in any of claims 1 to 4 and pharmaceutical ancillary substances.